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International Journal of Current Research
Vol. 10, Issue, 02, pp.65340-65343, February, 2018

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

IMPACT OF BIOPESTICIDE – *AZADIRACHTA INDICA* ON BIOCHEMICALS OF FRESH WATER CATFISH *HETEROPNEUSTES FOSSILIS*

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ARTICLE INFO

Article History:
Received 17th November, 2017
Received in revised form
02nd December, 2017
Accepted 22nd January, 2018
Published online 28th February, 2018

Key words:
Azadirachta indica,
Heteropneustes fossilis,
Sub-lethal concentrations,
Liver, Muscle and intestine.

ABSTRACT

The side-effect of *Azadirachta indica* (A. Juss) on certain biochemical parameters of a freshwater catfish, *Heteropneustes fossilis* was studied for a period of 24, 48, 72, and 96 hr, at sublethal concentration. During the exposure period the protein and glycogen content in the liver, muscle and intestine of treated fish showed decreasing trend.The alterations of these biochemical parameters can be effectively used as nonspecific biomarkers against plant extract toxicity stress and also help safer usage of plant extracts in aquaculture farms.

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Citation: Dr. Vaishali S. Panchwate, 2018. "Impact of biopesticide – *Azadirachta indica* on biochemicals of fresh water catfish *heteropneustes fossilis*", *International Journal of Current Research*, 10, (02), 65340-65343.

INTRODUCTION

Medicinal plants are part and parcel of human society to combat diseases, form the dawn of civilization. *Azadirachta indica*, A Juss (Syn. *Melia. Azadirachta*) is well known in India and its neighbouring countries for more than 2000 years as one of the most versatile medicinal plant having a wide spectrum of biological activity. *A Indica*. *A. Juss* and *M. azedarach* are two closely related species of Meliaceae. The former is popularly known as Indian *Neem* (Margosa tree) or Indian lilac, and the letter as the Persian lilac. *Neem* is hailed as a wonder tree "kalpavriksha" means miraculous tree for its versatile use. Products made from *Neem* trees have been used in India for over two millennia for their medicinal properties. *Neem* products are trusted by Ayurvedic practitioners to be anthelmintic, antifungal, antidiabetic, antibacterial, antiviral, contraceptive and sedative. It is considered a major component in Ayurvedic and Unani medicine and is particularly prescribed for skin diseases. *Neem* oil is also used for healthy hair, to improve liver function, detoxify the blood, and balance blood sugar levels. *Neem* leaves have also been used to treat skin diseases like eczema, psoriasis, etc. However, insufficient research has been done to assess the purported benefits of *Neem*.

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In adults, short-term use of *Neem* is safe, while long-term use may harm the kidneys or liver; in small children, *Neem* oil is toxic and can lead to death. *Neem* also causes miscarriages, infertility, and low sugar (Krishnan 2009) *Neem* oil is endowed with many medicinal properties and many *Neem* oil based traditional preparations used for pesticides, fungicides and also for treating many human and livestock disease. Therefore, *Neem* is a fascinating tree; it may usher in new era of pest control, provide millions of with inexpensive medicine, cut down rate of human population growth and restore soil productivity. Fishes and other aquatic organisms have been used as biomonitors or indicators of polluted or contaminated environments (Gabriel and Kparobo, 2003, Gabriel and George 2005). This is generally achieved through the measurement of biochemical, physiological and behvaioural responses of the organisms in question (Agbon and Omoniyi 2002, Tiwari and Singh 2006). Biochemical studies are good parameters which help to see the effect of toxicants on metabolism of fish (Ghosh 1986, Kajare *et al*. 2000). Biochemical changes in fishes exposed to various biopesticides have been documented (Ogueji and Auta 2007, Kori-Siakpere and Ubogu 2008, Mousa *et al.* 2008 Shalaby 2009). The alteration in biochemical contents in different tissues of fish due to toxic effects of different heavy metals and pesticides have been reported by number of workers (Gupta *et al.* 1987, Khan *et al.* 1992, James and Sampath 1995, Das *et al.* 1999, Khare and Singh 2002, Hadi *et al.* 2009). Accumulation of biopesticides in tissues produces many physiological and biochemical

changes in the fishes and freshwater fauna by influencing the activities of several enzymes and metabolites (Nagarathnamma and Ramamurthi 1982). Proteins are the building blocks for cellular and sub cellular structure and are present in all living cells. The protein plays a major role in the synthesis of microsomal detoxifying enzymes and helps to detoxify the toxicants when entering into the animals (Wilkinson 1976). Wigglesworth (1979) has stated that the fat body in insect is the main site for protein synthesis as well as the intermediating metabolism of amino acids, which are utilized for the production of hormones and enzymes and the composition of protein in the body as a whole may be greatly modified (Palanichamy *et al.*1989). Glycogen is an important nutrient reserve in animal tissue and it is used as an immediate energy source when required by any animals. Therefore, glycogen is an essential component of the normal metabolism (Thunberg and Manchester 1972). The glycogen content in liver, kidney and muscles is considered as one of the important sensitive biochemical indicators that reflects the various functional changes in the systems of an organism (Metelev *et al*. 1983). Tissue carbohydrates are affected by pesticide toxicity. Sharma and Mahajan (1983) showed that affected biochemical factors like blood glucose and liver and muscle glycogen. Vasanthi and Ramaswamy (1987) found a shift in the metabolic pathway and subsequent changes in glycogen mobilization in the tissues of *Sarotherodon mossambicus* exposed to pesticide.

MATERIALS AND METHODS

For the experiment fresh water catfish *Heteropneustes fossilis* were selected and divided into two groups with 10 fishes in each aquarium. Each group was exposed to sublethal concentration of the *Azadirachta indica* similar set up was also maintained as control. During sublethal studies, fish were fed by locally available marketed food. The animals were scarifies for optimal concentration of biopesticide for different exposure of 24, 48, 72 and 96 Hrs. For biochemical studies, fishes were scarified during the exposure period of 24, 48, 72 and 96 Hrs respectively. The toxicant was renewed after fixed period.

Biochemical Studies

For biochemical studies liver, muscle and intestine were dissected out, washed in chilled fish saline, blotted, weighted and homogenized in pestle and mortar. Homogenization was performed in different media as per the requirement of the techniques involved. Protein estimation was done according to the method of Lowry *et al.* (1951). Estimation of glycogen was estimated by by procedure of Montgomery (1957).

Statistical analysis

Data for the different parameters were expressed as mean (±SE). Multivariate linear regression models were used to assess the differences in the different parameters in either control or test groups. The observed results between different groups were compared using the Analysis of Variance (ANOVA).

OBSEVATIONS AND RESULTS

During the course of experiments, *Heteropneustes fossilis* was exposed to sub lethal concentration of *Azadirachta indica*. The mean (±SE) values mentioned biochemical parameter for selected tissues was observed to be significantly lower for the experimental groups compared with the controlled group. (Table 1 and Figure 1)

Table 1. Effect of *Azadirachta indica* on tissues Protein of *H. fossilis*

Sr .	Tissue	Control	24 Hr	48 Hr	72 Hr	96 Hr
1	Liver	103.1 ± 1.615	99.25 ± 1.848	96.75* ± 2.145	84.75** ± 1.091	82.87** ± 1.555
2	Muscle	112.5 ± 0.285	105.5** ± 0.935	103.75** ± 0.684	99.12** ± 1.359	92.99** ± 1.276
3	Intestine	89.12 ± 0.672	84.50 ± 2.178	78.24** ± 0.321	71.50** ± 1.191	67.11** ± 1.195

± SE, * Significant at P< 0.05, ** Significant at P< 0.01 and P< 0.05 both

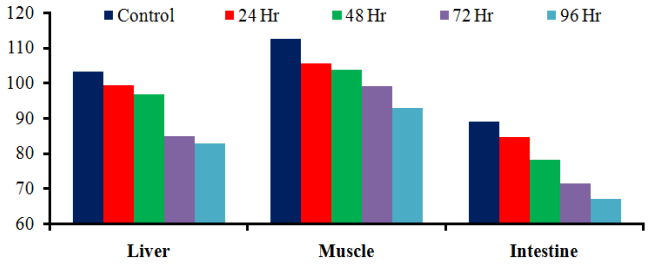

Fig.1. Effect of *Azadirachta indica* on tissues Protein of *H. fossilis*

During the course of experiments, *Heteropneustes fossilis* was exposed to sub lethal concentration of *Azadirachta indica*. The mean (±SE) values mentioned biochemical parameter for selected tissues was observed to be significantly lower for the experimental groups compared with the controlled group. (Table 2 and Fig.2)

Table 2. Effect of *Azadirachta indica* on tissue Glycogen of *H. fossilis*

Sr .	Tissue	Control	24 Hr	48 Hr	72 Hr	96 Hr
1	Liver	6.31 ± 0.008	5.49** ± 0.063	4.88** ± 0.124	4.70** ± 0.060	4.35** ± 0.053
2	Muscle	4.14 ± 0.073	3.57* ± 0.096	3.24** ± 0.090	2.92** ± 0.085	2.86** ± 0.126
3	Intestine	3.01 ± 0.050	2.46* ± 0.070	2.10** ± 0.020	1.91** ± 0.470	1.82** ± 0.016

± SE, * Significant at P< 0.05, ** Significant at P< 0.01 and P< 0.05 both

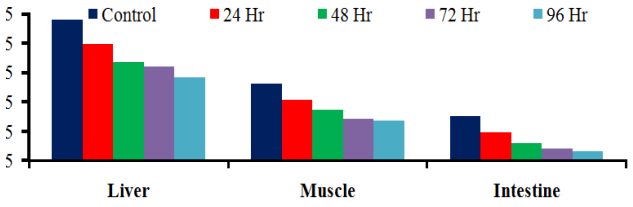

Fig.2. Effect of *Azadirachta indica* on tissue Glycogen of *H. fossilis*

DISCUSSION

In the present investigation, during treatment significant behavioural changes like increase in opercular movement, mucous secretion, erratic movement etc., were noticed. Tiwari and Singh (2003) also noticed similar behavioural changes in *Channa punctatus* exposed to *Nerium indicum* leaf extract. Carbohydrates are the primary and immediate source of energy (Tiwari and Singh, 2006); under str ess, carbohydrate reserves get depleted to meet energy demand. Depletion of glycogen may be due to direct utilization for energy generation, a

demand caused by aqueous extract-induced hypoxia (Kohli *et al.,* 1975). Glycogen the ultimate energy source, decreases, resulting in higher demand for carbohydrate and their precursors to keep the glycolytic and Krebs's cycles at sustained levels to cope with energy demands during stress condition. Reduction in glycogen level is thought to be the result of greater stress the organs experienced during the process of detoxification of active moieties and their metabolites. Liver glycogen levels are depleted during acute hypoxia or physical disturbances in the fish (Heath and Fritechard, 1965). In the present study the decrease of glycogen in liver, muscle and intestine may be due to direct utilization of energy generation, a demand caused by aqueous extract induced hypoxia. The depletion of protein fraction in liver, muscle and intestine might have been due to their degradation and possible utilization for metabolic purposes. During chronic period of stress, proteins act as a source of energy. Bradbury *et al.* (1987) pointed out that the decreased protein content might also be attributed to the destruction or necrosis of cells and consequent impairment in protein synthesis machinery. Tiwari and Singh (2006) reported that the decreases in protein level in the liver and muscle of fish exposed to neem extract might have resulted from high protein hydrolytic activity due to elevation of protease enzyme activity in both the tissues. In the present study the depletion of protein level in liver, muscle and intestine tissues during sublethal treatment might have resulted from their degradation and possible utilization of degraded products for metabolic purpos

Conclusion

The present study indicates that *A. indica* has caused significant alterations in protein and glycogen content of liver, muscle and intestine of fish *Heteropneustes fossilis.* So finally, it is doubtless that *Azadirachta indica* that is Neem is very beneficial plant because its products are believed to have several medicinal values. The Neem is one of the best pesticides which shows very less hazards to environment. But the present study on *Heteropneustes fossilis* and previous research made on the aquatic organisms especially fresh water fishes proved the hazardous effects of *Azadirachta indica* on stress management, behaviour, survival along with biochemistry, enzymology, structural and reproductive biology. So, in future there is need of more research to assess the purported benefits of Neem which is a *Kalpavriksha* for all the mankind.

Acknowledgements

This project was supported by Respeted Dr.V.T. Tantarpale, Vidya Bharti Collage, Amravati.

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